Reference: *Biol. Bull.*, **136**: 91–95. (February, 1969)

NATURAL AND SYNTHETIC MATERIALS WITH INSECT HORMONE ACTIVITY. 2. JUVENILE HORMONE ACTIVITY OF SOME DERIVATIVES OF FARNESENIC ACID

K. SLÁMA, M. ROMAŇUK AND F. ŠORM

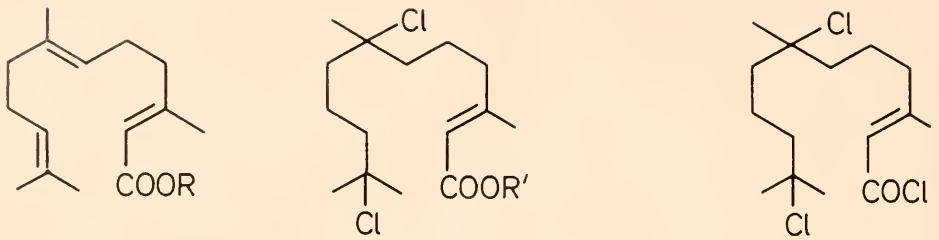
Institute of Entomology and the Institutes of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague

A number of substances with juvenile hormone activity have been isolated from natural sources (Bowers *et al.*, 1966; Černý *et al.*, 1967; Röller *et al.*, 1967; Schmialek, 1961) or prepared synthetically (Ayyar and Rao, 1967; Dahm *et al.*, 1967; Mori and Matsuji, 1967; Romaňuk *et al.*, 1967; Schmialek, 1963; Schneiderman *et al.*, 1965; Sláma *et al.*, 1968) during the past few years. Most of these substances are derived from terpenes of farnesane or bisabolane types. Law *et al.* (1966) discovered that the reaction between hydrogen chloride and an alcoholic solution of farnesenic acid produced a mixture of extremely active juvenile hormone analogues. The authors observed that the activity of the reaction products was dependent on the alcohol used, ethanol giving maximum activity.

It was later found (Romaňuk *et al.*, 1967) that for *Pyrrhocoris apterus* the most active components of this mixture were esters of farnesenic acid (I) in which the two double bonds were saturated by hydrogen chloride (VII-IX). Subsequently, in our search for new juvenile hormone analogues we have synthesized a number of farnesenic acid derivatives with pronounced changes in biological activity. In the present communication we give an account of the juvenile hormone activity of some selected derivatives when assayed on four species of insects.

Materials and Methods

Juvenile hormone activity was determined by topical assays on *Pyrrhocoris apterus* and *Dysdercus cingulatus* (Hemiptera, Pyrrhocoridae), on *Graphosoma italicum* (Hemiptera, Pentatomidae) and by injection assays on pupae of *Tenebrio molitor* (Coleoptera, Tenebrionidae). In the topical tests we applied the substances in 1 μl acetone to the abdominal tergites of freshly molted last instar larvae. In the *Tenebrio* assay, we injected the substances in 1 μl olive oil into freshly molted pupae. The activity was determined by the degree of inhibition of metamorphosis and was expressed in juvenile hormone units (Sláma, 1968). One unit indicates the amount of substance, in micrograms per specimen, which caused half-larval, half-adult intermediates (Hemiptera) or half-pupal, half-adult intermediates (*Tenebrio*). In Hemiptera the whole range of activity from zero (normal adults) to the maximum (supernumerary larvae) was realized with a ten-fold change in concentration. Thus, when one unit is determined as 0.05, it indicates that the compound shows a trace of activity at 0.01, medium activity at 0.05, and maximum activity at 0.1 μg per specimen. In *Tenebrio* the activity range from zero to maximum extended over 100- to 1000-fold



I R = H
II R = -CH_3
III R = -C_2H_5
IV R = 
V R = -$CH_2C_6H_5$

VI R' = H
VII R' = -CH_3
VIII R' = -C_2H_5
IX R' = -C_3H_7
X R' = -C_4H_9
XI R' = -C_4H_9(tert)

XII R' = -C_5H_{11}
XIII R' = -C_6H_{13}
XIV R' = 
XV R' = -$CH_2C_6H_5$
XVI R' = -$CH_2C_6H_4Br$

VIa

FIGURE 1.

changes in concentration. Serial dilution were used to determine the range of activity of each compound; then, assays within this concentration range permitted a more accurate determination of activity. If the compounds did not show any activity below 100 μg in topical assays or 1000 μg in injection assays, no further tests were performed.

The compounds (I)-(III) were prepared by conventional methods; (IV) and (V) were synthesized by alkylation of silver salts of farnesenic acid with cyclohexyl and benzyl iodide respectively. For the synthesis of esters (VII)-(XVI) we used crystalline *trans*-dihydrodichlorofarnesenic acid (VI) (*trans*-3,7,11-trimethyl-7,11-dichloro-2-dodecenic acid, m.p. 93–94° (Romaňuk and Šorm, 1968). The acid was prepared by bubbling gaseous hydrogen chloride through an acetic acid solution of farnesenic acid. It was then treated with thionyl chloride to produce the acid chloride (VIa) which, in turn, was reacted with various alcohols to give the corresponding *trans* dichloro-esters.

Farnesol and farnesyl methyl ether were commercial samples which were checked for purity by gas chromatography. Methyl 10,11-epoxyfarnesoate was made in a usual way by action of perphthalic acid on methyl farnesoate. The preparation of p-(dimethyl-hexyl) benzoic acid derivatives has already been described (Sláma *et al.*, 1968). The synthetic *d,l*-juvenile hormone was a gift from Dr. H. Röller. "Juvabione" (methyl todomatuate) was the natural product isolated from balsam fir wood (Černý *et al.*, 1967).

Results and Discussion

The results are summarized in Table I. Farnesenic acid (I) had generally low activity, one unit being in all cases much greater than 100 μg. Its methyl

and ethyl esters (II), (III) had low activity on the hemipterans (1 unit = 10–50 μg) and substantial activity for *Tenebrio* (1 unit = 5–10 μg). The cyclohexyl and benzyl farnesoates (IV), (V) again had lower activity. Addition of hydrogen chloride to the double bonds of farnesenic acid and its esters (VI)-(XVI) was followed by drastic changes in the juvenile hormone activity for the hemipterans but not for *Tenebrio*. Thus, the dihydrodichlorofarnesenic acid (VI) was about 100 times more active in the hemipterans than the original farnesenic acid (I); the activity of the dihydrodichloro compounds increased from the acid (VI) to the methyl ester (VII) and ethyl ester (VIII) where it attained its maximum. It decreased again with increasing number of carbon atoms in the ester radical from the propyl ester (IX) to the cyclohexyl ester (X) to (XIV). The aralkyl esters (XV), (XVI) were again slightly more active on *Pyrrhocoris* and *Dysdercus*. These data support the earlier observations of Law *et al.* (1966) who found that the reaction between hydrogen chloride and farnesenic acid produced the most active juvenile hormone materials in the presence of ethanol.

The most active compound we tested for juvenile hormone activity was the ethyl ester of *trans*-dihydrodichlorofarnesenic acid (VIII) which, when compared

TABLE 1

Juvenile hormone activity units (indicated by an amount of the substance in μg per specimen which causes half-larval or half-pupal adultoids)

	Topical assays on larvae of			Injections into pupae of *Tenebrio*
	Pyrrhocoris	*Dysdercus*	*Graphosoma*	
(I) Farnesenic acid (FA)	>100	>100	>100	>100
(II) FA methyl ester	10–50	50	50	5–10
(III) FA ethyl ester	50	30	50	5
(IV) FA cyclohexyl ester	50	100	100	100
(V) FA benzyl ester	100	100	100	1000
(VI) Dihydrodichloro farnesenic acid (DFA)	1	1	>100	>1000
(VII) DFA methyl ester	0.0008	0.01	30	1000
(VIII) DFA ethyl ester	0.0005	0.003	1	100
(IX) DFA propyl ester	0.005	0.008	100	1000
(X) DFA n-butyl ester	0.5	0.5	>100	>1000
(XI) DFA tert. butyl ester	0.1	0.05	>100	>1000
(XII) DFA amyl ester	3	0.5	>100	>1000
(XIII) DFA hexyl ester	4	1	>100	>1000
(XIV) DFA cyclohexyl ester	3	5	>100	>1000
(XV) DFA benzyl ester	0.4	0.2	>100	>1000
(XVI) DFA p-bromobenzyl ester	0.09	0.4	>100	>1000
Farnesol	>100	>100	>100	100
Farnesyl methyl ether	30	10	100	10
10,11-epoxymethylfarnesoate	3	1	10	10
d,l-juvenile hormone	0.5	0.1	1	0.5
"Juvabione" (methyl todomatuate)	3	1	>100	>1000
p-(1,5-dimethyl-hexyl) benzoic acid methyl ester	0.5	0.1	>100	>1000
p-(1,5-dimethyl-1,5-dichlorohexyl) benzoic acid methyl ester	0.3	0.04	>100	>1000

with ethylfarnesoate (III), is 10^5 times more active on *Pyrrhocoris,* 10^4 times more active on *Dysdercus,* and 50 times more active on *Graphosoma.* As little as 0.1 nanogram of the compound (VIII) applied to *Pyrrhocoris* larvae produced adultoids which were unable to survive and reproduce. Thus, the minimum effective concentration appears to be 2.5 μg per kilogram live weight, or 2.5 mg per metric ton of insects representing approximately 25 million larvae. Experiments in which the larvae were reared on a filter paper impregnated with substance (VIII) revealed that a dose of 1 μg per square meter of filter paper was still effective. In practical terms, this shows that 10 mg per hectare could in principle prevent development of *Pyrrhocoris.*

Unlike the hemipteran insects, the pupae of *Tenebrio* were quite insensitive to the esters of dihydrodichlorofarnesenic acid. Their activity, in fact, was significantly decreased when compared with the esters of farnesenic acid. Thus, the same chemical changes of the molecular structure which lead to 10^5-fold increase of juvenile hormone activity in one species (*Pyrrhocoris*) may be followed by considerable loss of activity when assayed on another species. According to the present evidence, differences in insect sensitivity to these compounds occur, not only between hemipterans and *Tenebrio,* but also among the hemipterans themselves. As seen in Table I, the larvae of *Graphosoma* (Pentatomidae) were about equally sensitive to the unsubstituted esters of farnesenic acid as were the larvae of *Pyrrhocoris* and *Dysdercus* (Pyrrhocoridae), but much less sensitive to the dihydrodichloro compounds. It will also be observed that "juvabione" and *p*-(dimethyl-hexyl) benzoic acid esters (Sláma *et al.,* 1968) act only on the pyrrhocorid bugs and not on the pentatomid bug. Due to the observed selective effects on different species we expect that some of the analogues with relatively low juvenile activity on hemipterans or *Tenebrio* may later appear to be quite active on certain other insects.

Acknowledgment is gratefully given to Professor C. M. Williams who provided helpful comments on the typescript and to Mrs. Pichlová and Mrs. Kadlecová for their technical assistance.

Summary

The juvenile hormone activity of both farnesenic acid esters and their dihydrodichloro derivatives increased from the methyl to the ethyl ester, then decreased again. In some hemipteran insects enormous increases in activity were obtained after addition of two hydrogen chloride molecules to the two double bonds of farnesenic acid esters. The same change in chemical structure considerably decreased the activity on *Tenebrio* pupae. On *Pyrrhocoris apterus,* the highest activity (at the 0.1 nanogram level) was obtained with the ethyl ester of *trans*-3,7,11-trimethyl-7,11-dichloro-2-dodecenic acid.

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